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Nitrogen Fixation (C_2H_2-reduction) after Clearcutting of a Scots Pine (*Pinus sylvestris* L.) Coniferous Forest

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Abstract. No obvious effect of clearcutting of a Scots pine coniferous forest on its total soil nitrogen fixation (C_2H_2-reduction) was found for the first three years. Differences in the distribution of nitrogenase activity due to removal of slash were, however, recorded in the top soil layers. As a consequence of surface drought, removal of slash reduced acetylene reduction activity (ARA) in the surface layers, while slash deposits temporarily stimulated ARA, possibly also by nutrient release. ARA measured in soil varied between 0 and 275 pmoles C_2H_4 h^{-1} g^{-1} dry weight during the three years following clearcutting. Highest ARA was recorded in the autumn. A comparison made three and a half years and ten years after clearcutting, respectively, showed that a mature Scots pine stand had higher ARA in the humus layer and the mineral soil than the two clearcut sites. Annual nitrogen fixation the first and second year after clearcutting was estimated at 0.2–0.4 kg N ha^{-1} $year^{-1}$. Of this amount, the main part was fixed in the mineral soil.

INTRODUCTION

Nitrogen input via biological nitrogen fixation to temperate coniferous forest ecosystems is low and has been estimated at 0.5–3 kg N ha^{-1} $year^{-1}$ (Paul, 1978; Rosén & Lindberg, 1980). However, even low additions of nitrogen may be of importance in these types of climax ecosystems, which have low N availability and in which the competition for available nitrogen is high (Gosz, 1981; Granhall, 1981). Occurring for perhaps 100 years, throughout the duration of a forest rotation, nitrogen fixation also contributes a significant proportion of nitrogen to the ecosystem.

Clearcutting of a mature coniferous forest inevitably causes great abiotic and biological changes in the soil system (Tamm et al., 1974), affecting both bacteria (Niemelä & Sundman, 1977; Lundgren, 1982) and fungi (Bååth, 1980). The mode of addition of organic carbon from roots and aboveground litter changes, and readily available energy in the form of dead roots and slash is made available to the microorganisms. Clearcutting also results in wider fluctuations of moisture and temperature, especially in the topsoil (Huhta, 1971).

The extra addition of litter in the form of dead roots and slash following clearcutting could be expected to enhance nitrogen fixation, since litter constitutes one of the most important energy sources for heterotrophic nitrogen fixation in forest soils (Seidler et al., 1972; Sharp, 1975; Granhall & Lindberg, 1978; Larsen et al., 1978; Roskoski, 1980). More light reaching the soil surface and the exposure of mineral soil by scarification might also stimulate the growth of cyanobacteria (Granhall & Lindberg, 1978).

In earlier investigations (Granhall & Lindberg, 1978) we compared a young (15-year-old) Scots pine forest stand with a 120-year-old stand, and found a different spatial distribution of nitrogen fixation in the two stands, although the total amounts fixed per annum were similar. As a consequence of soil scarification, the young stand developed cyanobacterial crusts on the soil surface, with high specific nitrogenase activity. In the mature stand, however, heterotrophic nitrogen fixation, especially in the rhizosphere, dominated. At what rate and to what extent these changes in pattern occur after clearcutting is not known.

The aim of the present work was to quantify and evaluate the pattern of nitrogen fixation after clearcutting of a coniferous forest in comparison with that of previously investigated, undisturbed stands (Granhall & Lindberg, 1978), and also to study the effects of removal or deposit of slash.

MATERIALS AND METHODS

Site description

The clearcut forest site Ivantjärnsheden Ih 0 in Jädraås, Central Sweden, is one of the sites investigated in the Swedish Coniferous Forest Project (SWECON). The soil texture is glaciofluvial sand and the soil profile is a weakly developed iron podsol. The site had a 100-year-old Scots pine (*Pinus sylvestris* L.) forest that was clearcut in

March 1976. The experimental area contained 12 blocks (20 × 20 m). After clearcutting, the slash was collected and evenly distributed in doubled amount on half of the blocks, the other half being left without slash. Occasionally, samples were taken from two nearby sites, a mature (120-year-old) Scots pine stand (Ih V) and a site (Ih I) that was clearcut in 1967. Further information about the sites and the overall experimental design is given in Axelsson & Bråkenhielm (1980).

Sampling procedure

At Ih 0, three 1 m^2 sampling plots, randomly chosen within six replicate blocks of each treatment (with or without slash), were used for soil sampling. At Ih I and V, six 1 m^2 sampling plots were used. Soil cores were taken with an auger. The components of the soil investigated were: (1) the S-layer, consisting of lichens and mosses, together with attached litter; (2) the humus layer (A_{01}/A_{02}), containing decomposing organic material and roots; and (3) the mineral soil (B-layer) from immediately below the humus layer to 10 cm underneath the latter. On one occasion the mineral soil to 40 cm below the humus layer was tested at Ih 0. The soil did not have a very well-developed bleached layer. Twigs and branches from the slash at Ih 0 were tested separately.

Nitrogenase activity was measured using the acetylene reduction technique. Subsamples for acetylene reduction measurement were prepared as described by Granhall & Lindberg (1978). Twigs and branches were incubated in 3-liter desiccators. Occasionally, soil samples and different slash components (needles, twigs and branches) were tested 24 hours after wetting. All incubations were carried out in the laboratory at 22±2°C for 48 hours, surface samples in the light as previously described (Granhall & Lindberg, 1978), while other samples were incubated in the dark. The ethylene formed during incubation in samples with acetylene (five from each sampling plot), and in controls without acetylene (five from each sampling plot), was measured using gas chromatography (Granhall & Lundgren, 1971). Acetylene reduction activity (ARA) was estimated by subtracting endogenous ethylene formation.

Estimation of annual nitrogen fixation

ARA values were extrapolated to annual nitrogen fixation, considering the weight of different litter components and soil layers down to 40 cm in the mineral soil, as determined within various subprojects of the Swedish Coniferous Forest Project. Temperature was adjusted to mean monthly air temperature using the Q_{10} relation of Granhall & Lindberg (1978). Since drought is an important limiting factor for ARA in the uppermost soil layers, only days with rain (0–30 $months^{-1}$), plus the following three days during May–October were regarded as having nitrogenase activity in the S- and humus layers. In the mineral soil, all days during this period were regarded as having nitrogenase activity. For comparison with earlier studies, a factor of 3 was used to convert ARA to nitrogen fixation.

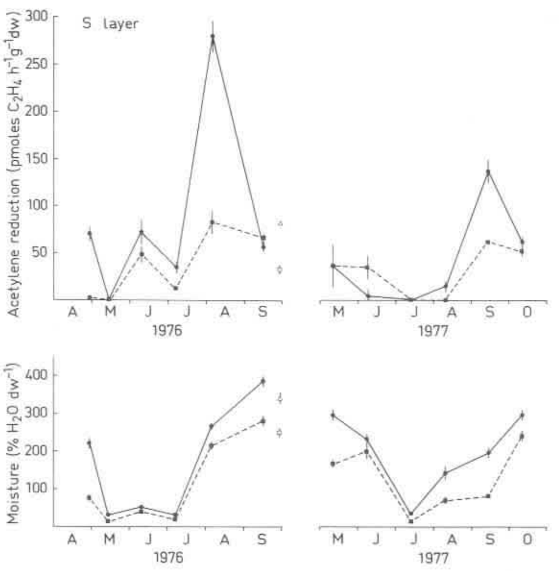

Fig. 1. Acetylene reduction and moisture in the S-layer after clearcutting. Soil samples from plots with slash (●—●) or without slash (■---■). A sampling before clearcutting (□) as compared with a nearby 120-year-old forested site (△) is included. Mean ± SE ($n=15$ for acetylene reduction and $n=30$ for moisture). Note different scale on y axis in Fig. 1 as compared with Figs. 3 and 4.

RESULTS

Nitrogenase activity in the S-layer

A pattern of low ARA during summer as well as low soil moisture, followed by a peak in ARA in August–September, was observed in the S-layer in both 1976 and 1977 (Fig. 1). Inhibition by low moisture during summer was therefore further examined on three different occasions by wetting soil samples

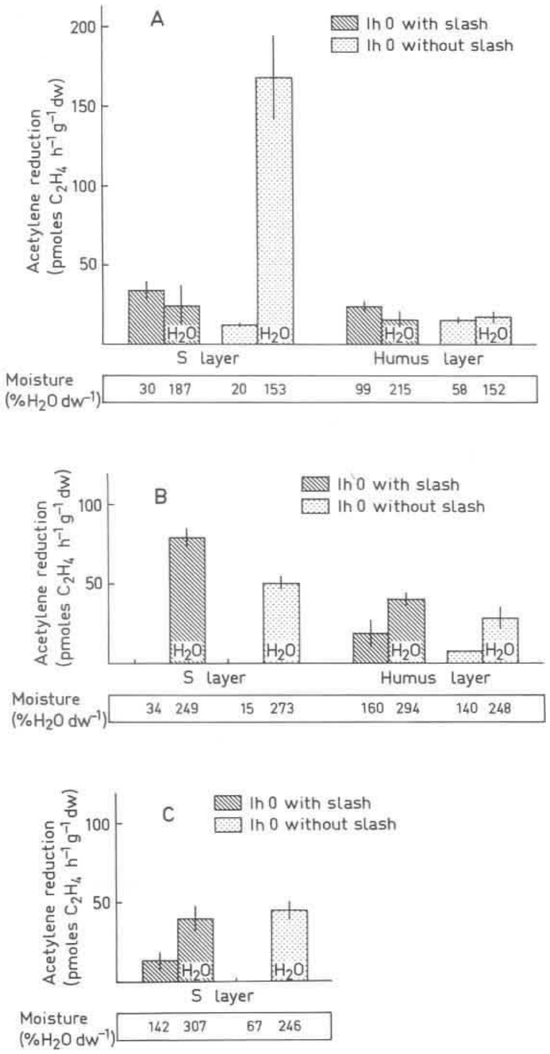

Fig. 2 A–C. Effect of wetting on acetylene reduction in the S-layer and humus layer at three different summer samplings. (A) July 7, 1976; (B) July 12, 1977; (C) August 9, 1977. Mean ± SE (acetylene reduction; n=15) or mean (moisture; n=30).

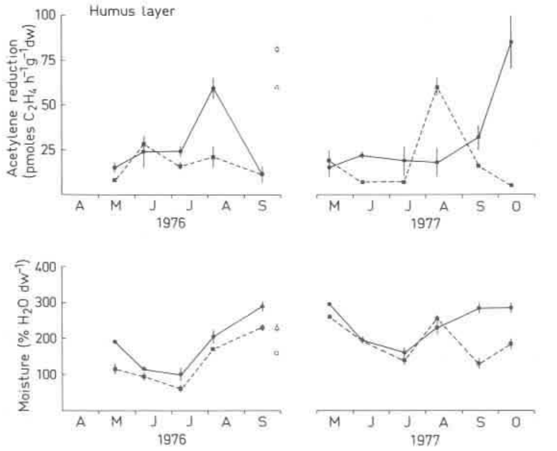

Fig. 3. Acetylene reduction and moisture in the humus layer after clearcutting. Same symbols as in Fig. 1.

from the top layers in the laboratory, thereby simulating rainfall in the field. In July 1976 only the S-layer without slash was clearly stimulated by wetting, but in July and August 1977 wetting stimulated ARA in both treatments (Fig. 2 A–C).

Samples from the slash treatment showed higher ARA values than those from plots without slash (Fig. 1). Differences were greatest in August 1976.

ARA was tested once (September 1975) before clearcutting of the 100-year-old stand (Ih 0), and at a nearby mature (120-year-old) stand (Ih V). The activities found were within the ranges found at Ih 0 after clearcutting both in the S-layer (Fig. 1) and in the humus layer (Fig. 3). Nitrogenase activity in stand Ih V has previously been investigated by Granhall & Lindberg (1978).

Nitrogenase activity in the humus layer

On a soil dry-weight basis, the activity in the humus layer was generally lower than in the S-layer, but was not severely inhibited by drought during summer (Fig. 3). ARA could, in one case out of two, be stimulated by adding water (Fig. 2 A, B). In 1976 there was little difference between slash and no-slash treatments, except in the August sampling, when slash plots showed higher ARA in analogy with the S-layer (Fig. 3).

In 1977 slash-plot values were rather constant until late autumn (October), when they peaked. Values for the no-slash treatment peaked in August, however, and were low in October.

Nitrogenase activity in the mineral soil

ARA in the upper 10 cm of the mineral soil (B-layer) was low and did not vary much between samplings and treatments over the period investigated (Fig. 4). Higher values were, however, noted in August 1976 for slash-plot samples, in line with the other two soil layers, and in May 1977 for no-slash-plot samples, unlike the case of the other layers at that sampling.

Nitrogenase activity in relation to soil depth was tested in September 1976. ARA could be detected down to 40 cm in the mineral soil (Fig. 5).

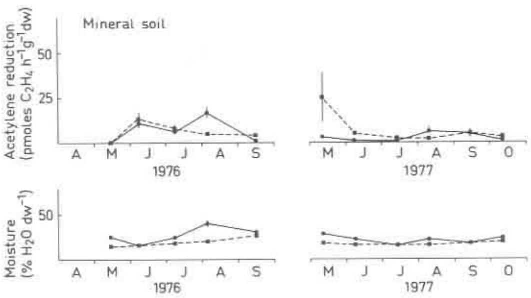

Fig. 4. Acetylene reduction and moisture in the mineral soil (B-layer) after clearcutting. Same symbols as in Fig. 1.

Nitrogenase activity in the third year after clearcutting

Three samplings in 1978 yielded ARA values within the ranges 15–61, 10–51 and 1–10 pmoles $C_2H_4\ h^{-1}\ g^{-1}$ dw in the S-layer, humus layer and mineral soil respectively. These ranges of activity do not differ from those found in the two preceding years.

ARA of slash

This was tested in September 1977, when the slash was 1.5 years old. The slash was very dry when collected; accordingly, it was also tested after wetting. All components except very wet needle litter had low ARA (Table 1).

Comparison between sites

A comparison between the recently clearcut area (Ih 0), a 12-year-old clearcut area (Ih I) and a mature Scots pine stand (Ih V) was made in September 1979, 3.5 years after the clearcutting of Ih 0. In the S-layer, ARA was highest at Ih 0 under slash, and lowest in the old clearcut area (Fig. 6). In the humus layer, and in the mineral soil, the mature stand had the highest activities; the values for the other stands were low.

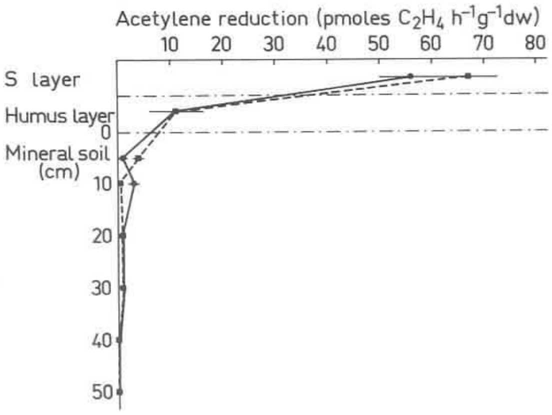

Fig. 5. Variation in acetylene reduction with depth. Soil samples from plots with slash (●—●) or without slash (■---■). Sampling date September 15, 1976. Mean ± SE (*n*=5).

Endogenous ethylene formation

Endogenous ethylene formation occurred in the S-layer (0–324 pmoles $C_2H_4\ h^{-1}\ g^{-1}$ dw) and the humus layer (0–113 pmoles $C_2H_4\ h^{-1}\ g^{-1}$ dw) but not in the mineral soil (cf. Lindberg et al., 1979). This makes it more difficult to interpret data from the S- and humus layers, since the presence of acetylene in ARA assays may influence endogenous ethylene metabolism, thereby causing overestimation of acetylene reduction (Witty, 1979; Nohrstedt, 1983).

DISCUSSION

Seasonal variations in the different soil layers

Fluctuations in nitrogenase activity at the clearcut site Ih 0 were greatest in the S-layer, followed by

Table 1. *Acetylene reduction in different slash components at different moisture levels. n=10 for needle litter and 2 for twigs and branches*

	Acetylene reduction (pmoles $C_2H_4\ h^{-1}\ g^{-1}$ dw)		Moisture (% $H_2O\ dw^{-1}$)	
Slash component	Mean	SE	Mean	SE
Needle litter	2.8	0.3	12	0.2
Needle litter, sprayed	0		33	0.4
Needle litter, soaked	53.7	9.9	187	3.7
Twigs (dia. 2–5 mm)	0		11	
Twigs, sprayed	6.0		25	
Branches (dia. 7–30 mm)	0		15	
Branches, sprayed	1.8		20	

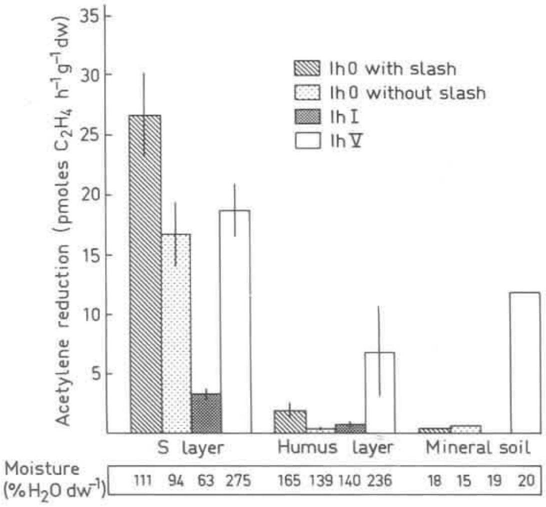

Fig. 6. Difference in acetylene reduction between a 3.5-year-old clearcut (Ih 0), a 12-year-old clearcut (Ih I) and a mature (120-year-old) Scots pine forest (Ih V). Sampling date September 12, 1979. Mean ± SE (n=6 for Ih 0 and n=12 for Ih I and Ih V), except for mineral soil which represents a composite sample of six subsamples.

the humus layer, while in the mineral soil (B-layer) values were rather constant throughout the investigation period. This is in keeping with the respective size of fluctuations in soil moisture and temperature (P. E. Jansson, pers. comm.) in the different layers. In the S-layer, ARA was inhibited by summer drought (Fig. 1) and could be stimulated by adding water (Fig. 2 A–C). Lundgren (1982) found a significant correlation between bacterial numbers and moisture at Ih 0, and Granhall & Lindberg (1978) found a high correlation between moisture and ARA of soil layers in three different nearby Scots pine stands.

Highest ARA was detected in the autumn. The same seasonal pattern in the upper soil layers and the absence of regular seasonal variations in the lower soil layers were also noted for FDA-active fungal hyphae at Ih 0 (Bååth & Söderström, 1982), indicating that ARA correlated with microbial activity in general.

Effects of slash

As long as the fresh slash is not in close contact with the soil, it is greatly exposed to drought, and it is probable that ARA in the slash itself may occur only in connection with rain. Even then there does not seem to be a high potential for acetylene reduction in the slash (Table 1). Needle litter has previously been shown by Granhall & Lindberg (1978) and Lindberg & Berg (1982) to have low ARA. Older, more highly decayed twigs and branches in contact with the soil do, however, show higher ARA values (Granhall & Lindberg, 1978), and these slash components might therefore become more important in a longer time perspective than the period covered by this investigation.

Activities were, with few exceptions, higher in the soil under slash plots than in no-slash plots. This agrees with the finding that bacterial biomass was higher under slash (Lundgren, 1982). The differences between the two treatments may partly be explained by the higher soil moisture content in slash plots (Figs. 1, 3, 4). Lundgren (1982) reported a mean moisture content of 280% H_2O dw^{-1} in slash plots, compared with 200% in no-slash plots. Removal of slash resulted in faster decomposition in the topsoil. A decrease in soil organic matter and plant nutrients as compared with slash plots was already apparent one and a half years after clearcutting (Staaf & Berg, 1980). Since ARA is correlated to the organic matter content of the soil (Granhall & Lindberg, 1978), this impoverishment could have resulted in lower ARA.

The peak in ARA in slash plots in August 1976, 5 months after clearcutting, was obvious in all three sampled layers and may be attributed to easily metabolized compounds leached from the slash. Leached substances from Scots pine needles are quickly metabolized by aerobic microorganisms (Nykvist, 1963).

In the mineral soil, activities were mostly low and constant, with no obvious differences between treatments. It seems possible that slash treatment had little or no effect on ARA in the soil below the humus layer. This agrees with the findings obtained for bacterial biomass (Lundgren, 1982).

Differences between stands

Tests performed on one occasion, 3.5 years after the clearcutting of Ih 0, showed that the mature stand had higher ARA in the humus layer and mineral soil than the clearcut stands (Fig. 6). An explanation may be that the mature stand has a more active rhizosphere microflora in these layers than the clearcut sites. Granhall & Lindberg (1978) concluded that nitrogen fixation in decaying wood, including the rhizosphere, is an important source of fixed nitrogen in a mature Scots pine forest. The

Table 2. *Estimated annual nitrogen fixation after clearcutting*

	g N ha^{-1} year^{-1}						
	S-layer	Humus layer	Mineral soil 0–40 cm	Slash	Woody litter (stumps and pine roots)		Total
1976							
without slash	2.4	2.4	238	0	71.9	2.4	315
with slash	5.2	3.7	268	0.4	71.9	2.4	349
1977							
without slash	1.5	2.6	319	0	82.0	3.8	405
with slash	3.4	5.0	121	0.4	82.0	3.8	212

old clearcut stand (Ih I) had low ARA in the S-layer, as well as low bacterial and fungal biomass (Bååth, 1980; Lundgren, 1982). The S-layer at Ih I has been adversely affected by summer frosts, and vegetation was very sparse (Axelsson & Bråkenhielm, 1980). It is probable that ARA in this layer will not reappear until reforestation allows a new S-layer to develop.

We never detected any cyanobacteria, either free-living or in the form of crusts, in the investigated sites. It is probable that such crusts only appear after damage to the topsoil, which causes exposure of the mineral soil (Granhall & Lindberg, 1978). This did not take place at Ih 0, but may occur after planting, since soil scarification is a recommended silvicultural practice for reforestation of Scots pine on sandy soils in this area.

Annual nitrogen fixation

In the S-layer, moisture was found to be an important limiting factor during the summer. Since moisture can vary greatly over short periods, so does ARA. An extrapolation to annual nitrogen fixation should therefore be seen only as an approximation of actual fixation. Such an approximation is useful for comparison with estimations of nitrogen fixation in other stands in this area. Granhall & Lindberg (1980) calculated annual nitrogen fixation in the soil down to 30 cm in the mineral soil of the nearby 120-year-old stand (Ih V) at 0.17 kg N ha^{-1} year^{-1} and in a young stand (Ih II) at 0.20 kg N ha^{-1} year^{-1}. A calculation for Ih 0 (see Materials and Methods) yields fixation between 0.2 and 0.4 kg N ha^{-1} year^{-1} for the topsoil down to 40 cm in the mineral soil (Table 2), which is essentially the same as in the other stands, indicating no major changes in input by biological nitrogen fixation. In spite of low specific activity, 57–79% of this amount was fixed in the mineral soil. The contribution from the organogenic layers (except woody litter) never exceeded 4%.

Soil samples from slash plots generally gave higher values than no-slash soil samples in both the S- and the humus layers, although the contribution of these layers to overall fixation was small (Table 2). In the mineral soil there was no great difference in fixation between the two treatments and, since the immediate contribution of the slash itself to annual nitrogen fixation seems low, the effect of slash treatment on total fixation appears relatively small during the first few years after clearcutting.

Although significant changes in the composition of microbial biomass as a consequence of clearcutting have been recorded (Bååth, 1980; Bååth & Söderström, 1982; Lundgren, 1982), the above results show that no major changes in total nitrogen fixation occurred during the three years following clearcutting. Judging from the comparison with the old clearcut area, it seems likely, however, that nitrogen fixation will decrease until planting and reforestation allow a different nitrogen fixation pattern to occur.

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